The Bottlenose Dolphin (*Tursiops truncatus*) as a Model to Understand Variation in Stress and Reproductive Hormone Measures in Relation to Sampling Matrix, Demographics, and Environmental Factors

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LONG-TERM GOALS

Our overarching goal is to develop indicators and methods to quantify chronic stress in bottlenose dolphins. Much research has focused on the stimuli which induce stress in marine mammals, as well as the hormonal mediators of the stress response. Stress may be induced by a variety factors, including noise, pollutant or toxin exposure, presence of predators, loss of prey, and/or habitat changes. The stress response is complex and difficult to study experimentally in marine mammals due to ethical and logistical considerations, but has been well characterized in other laboratory mammal species. In mammals as well as other vertebrates, the stress response has two modes of operation. The fast mode involves the rapid release of fast-acting agents such catecholamines by the medulla which drive the fight-or-flight response, enhancing vigilance, alertness, arousal and attention. The catecholamines in turn play a major role in excitation of the hypothalamic-pituitary-adrenal (HPA) axis, initiating a hormonal cascade which culminates in stimulation of the adrenal cortex to secrete glucocorticoids (GCs). The delayed but more sustained response driven by GCs coordinates brain and body functions to cope with stress and facilitate recovery, adaptation and re-establishment of homeostasis. These functions include mobilization of substrates for energy metabolism, suppression of immune and inflammatory reactions, and inhibition of bone and muscle growth. Studies of both captive and free-ranging individuals support the existence of these same stress response pathways in marine mammals.

While the HPA axis and physiological processes driven by the GCs are essential for an individual's ability to respond and adapt to stress, prolonged stimulation can overly burden the body's regulatory systems and induce deleterious effects. Prolonged elevation of GC hormones can lead to chronic immune suppression and inhibition of other energy expending hormonal systems, including disruption of reproductive function along the hypothalamo-pituitary-gonadal axis, all of which may cumulatively lead to decreased survival and/or inability to reproduce. For this reason, developing indicators and methods to quantify chronic stress in marine mammals is essential for understanding risks and long-term consequences for populations.

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OBJECTIVES

Using the bottlenose dolphin as a model species, specific objectives for this project are:

- Determine correlation of hormone measures (cortisol, T3, T4, FT4, reproductive hormones) between blood and blubber
- Develop a comprehensive understanding of factors that influence stress hormone levels and establish reference intervals for blood and blubber measurements, determining necessary stratifications by sex, age and/or sampling season.
- Examine relationships among the various hormone measures, and conduct preliminary screening analysis to examine potential relationships between the stress hormones and other health measures including immune function.

APPROACH

The Chicago Zoological Society's (CZS) "natural laboratory" situation in Sarasota Bay, Florida, where a resident population of bottlenose dolphins has been studied for more than 40 years, including demographic studies, capture-release health assessments, remote biopsy sampling, behavioral studies, and characterization of stress and stressors, provides unique opportunities to address questions related to stress. Serum hormones (cortisol, aldosterone, thyroid and reproductive hormones) have been routinely measured in blood as part of the health assessment which also includes a complete physical examination, morphometric measurements, hearing test, and sampling of blood, skin, blubber, urine, feces and blowhole swab. The collected tissue samples are analyzed for a broad suite of diagnostics.

Using the Sarasota model, we have more recently initiated studies targeting populations in heavily impacted coastal sites to gain an understanding of the effects of biological and chemical stressors on dolphin population health. Capture-release studies have been conducted in the Florida Panhandle where we are investigating the effects of chronic algal toxin exposure, and along the Georgia coast where we are examining the impacts of high exposure to legacy chemical contaminants. In all of these capture-release projects, we have collected data on reproductive, and thyroid hormones, as well as indicators of functional immunity (*e.g,* lymphocyte proliferation, neutrophil and monocyte phagocytosis), all measured simultaneously from the same individuals and processed by the same laboratories to ensure inter-study comparability.

We are meeting our proposed objectives by leveraging this existing collection of data and expanding sampling to include additional reference populations. One of the additional reference populations in a northern latitude site will be sampled across seasons to elucidate potential seasonal variation in blubber hormone measures.

The project is a collaborative effort, in conjunction with Dr. Lori Schwacke and Eric Zolman, NOAA/ National Ocean Service, Hollings Marine Laboratory, Dr. Nicholas Kellar, NOAA/National Marine Fisheries Service (NMFS), Southwest Fisheries Science Center, Dr. Patricia Rosel, NOAA/NMFS Southeast Fisheries Science Center, Dr. Stephanie Venn-Watson, National Marine Mammal Foundation, and Dr. Teresa Rowles, NOAA/NMFS, Office of Protected Resources. Dr. Schwacke serves as PI for a matching project.

WORK COMPLETED

Much of the planned field work for the CZS portion of this joint project has been completed. Matched blood and bluber samples were collected from dolphins in Sarasota Bay during May 2009 (n=20), May 2010 (n=10), and May 2011 (n=15). More than 2,100 hormone measures previously obtained for Sarasota Bay dolphins will also be applied to this project. In addition, the CZS team assisted NOAA with collecting matched blood and blubber samples from bottlenose dolphins along the Georgia coast in August 2009 (n=24), and from Barataria Bay, Louisiana in August 2011 (n= ~20). Additional samples will be collected in Sarasota Bay during May 2012.

RESULTS

This project has just begun, so all analyses remain to be completed.

IMPACT/APPLICATIONS

We expect to better define the range of natural variability of stress hormones for bottlenose dolphins, as well as stress hormone responses to a variety of natural and anthropogenic stressors. By examining relationships between stress hormones in blood and blubber, we hope to enhance the utility of remote blubber biopsy sampling as a tool for measuring stress hormones, and reduce the need for dolphin capture-release -- a stressful, expensive, and logistically complex activity – to obtain stress hormone measures. We will also examine potential relationships between stress hormone measures and longer-term dolphin health indicators in order to identify potenial impacts of stress.

RELATED PROJECTS

A matching project is being conducted under the leadership of Dr. Lori Schwacke of the NOAA/National Ocean Service Hollings Marine Laboratory (Project No. N0001411IP20085). The current project provides samples from Sarasota Bay bottlenose dolphins and assists with the projects conducted primarily by Dr. Schwacke and her team, in Barataria Bay, St. Joseph Bay, and coastal Georgia. Data analyses will be performed jointly.